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Myocyte Hypertrophy in the Transplanted Heart
A Morphometric Analysis

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In order to better define long-term changes in the transplanted heart with respect to the effects of cyclosporine and the ischemic time of the donor heart, endomyocardial biopsies were examined ultrastructurally from 20 cardiac transplant recipients three years posttransplantation. The biopsies were divided into four groups of five based on the donor heart ischemic time in "on-site" versus "distantly procured" hearts and on the immunosuppression protocol: group A: "on site" donor hearts and cyclosporine-based immunosuppression; group B: "on site" donor hearts with conventional immunosuppression (azathioprine-based immunosuppression without cyclosporine); group C: distantly procured donor hearts treated with cyclosporine; and group D: distantly procured donor hearts treated with conventional immunosuppression. All four groups showed a significant increase in the average width of myocytes when compared with normal myocardium, (group A, $P<0.05$; groups B, C, D, $P<0.01$). Also, there was a significant difference between the average widths of myocytes from on-site donor hearts and distantly procured donor hearts ($P<0.04$). There was no significant difference between the average myocyte widths of groups treated with cyclosporine and those with conventional immunosuppression.

This study shows that despite the hypertension induced by cyclosporine, myocyte hypertrophy at 3 years posttransplantation does not appear to be significantly greater than in patients treated with conventional immunosuppression. Distantly procured donor hearts have more hypertrophy. Due to the increasing evidence that cardiac hypertrophy per se may predispose to serious ventricular arrhythmias, this study supports the use of on-site as opposed to distantly procured donor hearts.

Three hundred and seventy cardiac transplants were performed at Stanford University Medical Center between 1968 and January, 1986. During this period the one-year survival increased from 22% to 88%, and 50% of cardiac recipients are now expected to live 5 years or longer (1, 2). The longest survivor is now 16 years posttransplantation. Because the long-term effects of cardiac transplantation are as yet unknown, and because long-term survivors may develop complications, these patients still undergo routine endomyocardial biopsy four times a year, and at least one of these is performed at Stanford as

part of an annual examination (3, 4). Therefore, we have had the opportunity to examine the morphology of the transplanted heart serially and have observed two consistent features: all the hearts show some degree of myocyte hypertrophy and an increase in interstitial fibrous tissue. These features have also been present at autopsy (e.g., the average heart weight at autopsy greater than one year posttransplantation is approximately 450 g irrespective of donor age). The etiology of these processes has been unclear and of little concern. However, now that it is becoming apparent that the mere presence of cardiac hypertrophy may be a risk factor for malignant ventricular arrhythmias (5–8) its prevention is desirable.

In this study we examined the role of two possible contributing factors to the development of myocyte hypertrophy in transplant recipients: the ischemic interval of the donor heart at the time of transplantation and the use of cyclosporine for immunosuppression, since this is known to cause hypertension (3).

MATERIALS AND METHODS

The myocardium from twenty cardiac recipients who underwent endomyocardial biopsy three years after transplantation was studied. The biopsy material was selected to provide four groups of five patients each. Group A consisted of myocardium from five patients treated with cyclosporine who had received hearts from on site donors with a mean ischemic time of 68 min. Group B consisted of myocardium from five patients treated with azathioprine and prednisone who had received an on-site-donor heart with a mean ischemic time of 43 min. Group C consisted of myocardium from five patients treated with cyclosporine, but who had received distantly procured donor hearts with a mean ischemic time of 153 min. Group D consisted of myocardium from five patients treated with azathioprine and prednisone who had received distantly procured hearts with a mean ischemic time of 149 min. The ages of the recipients and the donors are listed in Table 1. Myocardium from biopsies of ten young, heart-disease-free cardiac donors served as controls (group E).

All the study specimens examined were from right ventricular endomyocardial biopsies obtained approximately three years after transplantation utilizing the Stanford-Caves bioptome. The procedure for percutaneous endomyocardial biopsies at Stanford has been previously described (9, 10). The control biopsies were transatrial right ventricular endomyocardial biopsies obtained at the time of transplantation. The tissue was fixed immediately in 2.5% gluteraldehyde and 2% paraformaldehyde in 0.1 M sodium cacodylate buffer (pH 7.2). After fixation, the tissue was washed with several changes of cold buffer, post-fixed with cold 2% osmium tetroxide, stained en bloc with 2% uranyl acetate, and embedded in epoxy resin. Semithin sections of epon-embedded tissue were cut with an LKB II ultratome and stained with toluidine blue for screening and selection. Ultrathin sections were stained with lead citrate for 1 min. The tissue was then examined with a Phillip's EM 201 electron microscope.

The morphometric study was done using planimetry according to

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the computerized method in electron microscopy, and the objects to be measured were single-time traced with a digitizer. Low magnification electron micrographs were taken of ten myocytes cut in longitudinal section from each of the study patients. The first ten cells in which a nucleus with at least one nucleolus could be seen were chosen. Microscope magnification was calibrated with a diffraction grating replica, and photographic prints were enlarged to a final magnification of 4000. The entire width of each myocyte along the portion of its length that included the nucleus was contained within the print. One high-magnification electron micrograph of a group of perinuclear mitochondria was taken for each myocyte, and the final photographic print enlarged to a magnification of 14,200.

Measurements were made with a Micro-Comp morphometric system consisting of an IBM PC computer with a monitor and printer, a Bausch and Lomb Hipad digitizer, and a Micro-Comp interface card and program diskettes. This program permits several types of measurements—including area, perimeter, form factor, and length of objects in micrographs—as well as storage of data and statistical analysis. Area and length of myocytes, area and form factor of nuclei, and area of mitochondria were measured in this study.

The average width of each myocyte was calculated by dividing the available area of the myocyte area by its length. In ten myocytes (group A: 1; group B: 3; group C: 2; group D: 4), two separate nuclear profiles were seen close together, and their profiles were estimated together and their areas and form factors were calculated. Another two myocytes (groups A and B) contained two nuclei that were separated by some distance. In these cases, only the one of the two nuclei was measured. The form factor is derived from area and perimeter (form factor = 4π # [area]/[perimeter]^2), therefore a reduced form factor signifies an increased size and increased crenation of the nuclear envelope.

Mitochondria were chosen for measurement by a systematic sampling method. A series of parallel lines, each separated by 2 cm, was placed over the high-magnification micrographs. Beginning at one side, the first line was followed from one end to the other, and the first 5 mitochondria intersected by the line were chosen for measurement. If necessary, the second line was used, and so on, until 5 mitochondria intersected by the line were chosen for measurement.

The morphometric measurements were made in a blind manner without knowledge of the origin of the groups or electron microscopic preparations. The mean values of each group, including the normal hearts, were compared using the student's t test.

RESULTS

A summary of the results of the morphometric analysis is presented in Table 2.

TABLE 1. Cardiac transplant recipient and donor age (years; mean ± SD)

Group	Donor	Recipient
A	19.8±7.0	36.8±4.7
B	24.0±10.6	42.0±6.8
C	20.6±5.0	23.0±11.3
D	23.4±6.3	38.2±12.6
E (control)	25.3±8.7	

Group A: on-site donor hearts treated with cyclosporine. The endomyocardial biopsies from the on-site donor hearts subsequently treated with cyclosporine showed a significant increase in myocardial fiber diameter (19.11±1.14 μm) when compared with the normal myocardium ($P<0.05$). The area of the nuclei was 40.85±3.60 μm^2 and did not show any significant difference when compared with that of the controls. However, the nuclear form factor (0.389±0.022) was significantly smaller than that of the controls ($P<0.05$) and the mitochondrial area (0.385± 0.013 μm^2) was significantly increased ($P<0.05$).

Group B: on-site donor hearts treated with conventional immunosuppression. The myocyte width (20.69±0.86 μm) showed a significant increase when compared with that of the normal heart ($P<0.001$). There was no significant difference, however, between groups A and B. The nuclear area (35.10±1.93 μm^2) was decreased and significantly different from the normal hearts ($P<0.001$). The nuclear form factor (0.384±0.018) also showed a significant difference from the controls ($P<0.05$). The mitochondrial area (0.465±0.017 μm^2) was significantly larger than the controls ($P<0.001$) and that of group A ($P<0.001$).

Group C: distantly procured donor hearts treated with cyclosporine. The myocyte width was significantly increased at 23.46±0.98 μm when compared with those of the normal hearts and groups A and B. (control: $P<0.001$, group A: $P<0.001$, group B: $P<0.05$). The nuclear area was 45.81±3.27 μm^2, and there was no significant difference from the control group. However, the nuclear form factor (0.383±0.022) was significantly smaller than that of the normal heart ($P<0.05$). There were no significant differences from those of groups A and B, except when the nuclear area was compared with that of group B ($P<0.01$). The mitochondrial area (0.414±0.14 μm^2) showed a significant difference from controls ($P<0.001$), and group B ($P<0.05$), but not from group A.

Group D: distantly procured donor hearts treated with conventional immunosuppression. The width of myocardial fibers was 23.51±0.99 μm and significantly different from those of other groups except group C (control: $P<0.001$, group A: $P<0.01$, group B: $P<0.05$). The nuclear area was 49.52±3.00 μm^2 and showed a significant difference only when compared with that of group B ($P<0.001$). The nuclear form factor was 0.344±0.019 and showed no significant difference from those of other groups except when compared with that of the normal hearts ($P<0.001$). The mitochondrial area (0.427±0.064 μm^2) showed a significant difference from those of controls ($P<0.001$) and group A ($P<0.05$), but not from those of groups B and C.

In summary, all four groups showed a significant increase in the width of myocardial fibers when compared with those from normal hearts. Also, there was a significant difference between the average myocyte widths of on-site donor hearts and dis-

TABLE 2. Summary of morphometric measurements in cardiac transplant recipients three years posttransplantation compared with controls; values shown are mean ± SE

	A: CsA (on site)	B: Aza (on site)	C: CsA (distant)	D: Aza (distant)	E (control)
Myocyte width (μm)	19.11 ± 1.14[b]	20.69 ± 0.86[d]	23.46 ± 0.98[d]	23.51 ± 0.99[d]	16.76 ± 0.51
Nuclear area (μm^2)	40.85 ± 3.60[c]	35.10 ± 1.93[d]	45.81 ± 3.27[c]	49.52 ± 3.00[c]	45.21 ± 1.80
Nuclear form factor	.389 ± 0.22[b]	.384 ± .018[b]	.383 ± .022[b]	.344 ± .019[d]	.436 ± .013
Mitochondria area (μm^2)	.385 ± .013[b]	.465 ± .017[d]	.414 ± .014[d]	.427 ± .014[d]	.349 ± .008

[a] CsA: cyclosporine-based immunosuppression; Aza: azathioprine-based immunosuppression.
[b] $P<0.05$.
[c] $P>0.05$.
[d] $P<0.001$.

tantly procured donor hearts. The average myocyte width, however, did not show any significant difference between the groups treated with cyclosporine and those treated with conventional immunosuppression. The nuclear area of group B was significantly smaller than the other groups, including the control group, but there was no significant difference among the other groups. The nuclear form factors of all four groups were reduced and showed significant differences when compared with that of the normal hearts. The mitochondrial areas of all four groups were significantly larger than that of the controls.

DISCUSSION

For longer than ten years it has been empirically noted that the hearts of long-term cardiac allograft survivors show the presence of hypertrophy, whether examined at autopsy or endomyocardial biopsy. However, this observation has never been quantitated nor its etiology examined. The present study morphometrically analyzed the myocardial cells from patients three years posttransplantation and confirmed that, indeed, there is a significant increase in myocyte width posttransplantation, irrespective of the ischemic time of the donor heart or post-surgical immunosuppression therapy. Differences demonstrated in myocyte width between the groups of patients analyzed, moreover, shed light on possible reasons for this phenomenon.

Ventricular hypertrophy can be idiopathic, as occurs in a cardiomyopathy; it may be secondary to an increase in preload or afterload; it may be due to an impairment of pump function; or it may be due to a combination of these factors (5, 6). Several factors may be postulated as contributing to the myocardial hypertrophy observed in this study. Cyclosporine may result in increasing afterload by causing systemic arterial hypertension, which occurs in greater than 95% of patients and is frequently severe (3). In contrast, on conventional immunosuppression hypertension is infrequent and tends to occur only in older recipients with superimposed generalized atherosclerosis (3). Cyclosporine may also impair cardiac pump function by causing a fine interstitial myocardial fibrosis (11, 12). However, we found no significant difference in the myocyte width of cyclosporine and conventionally immunosuppressed patients making cyclosporine an unlikely cause of the hypertrophy.

The results of our study, in which the width of myocardial fibers was significantly greater in the distantly procured hearts, suggest that the ischemic time of the donor heart is an important factor in the development of the hypertrophy. In a previous report on the ultrastructural changes that occur after 3 hr ischemic time in distantly procured hearts, it was demonstrated that human hearts show significant ultrastructural pathology with this amount of ischemia that is even worse after reperfusion (13). We postulate that the prolonged ischemia that occurs during transportation of the distantly procured donor hearts and reperfusion injury eventually results in the loss of some myocytes from focal ischemic damage. With repair, there is then compensatory hypertrophy in the remaining myocytes, in much the same way as occlusive coronary artery atherosclerosis can result in myocyte hypertrophy.

Other factors that may impair cardiac function—and, therefore, stimulate the myocardium to hypertrophy in all transplant recipients—are the more patchy areas of fibrosis resulting from healed episodes of rejection; ongoing ischemia as a result of graft arteriosclerosis; and pericardial constriction. However,

these factors were not specifically addressed in this study. As all of our endomyocardial biopsies were from the right ventricle, the effects of superimposed pulmonary vascular disease may also have contributed to the hypertrophy. However, none of these patients had significant pulmonary hypertension prior to transplantation and there is no clinical or hemodynamic evidence that heart transplantation per se increases right ventricular preload or afterload. Furthermore, although this study utilized only right ventricular myocardium—at autopsy the hypertrophy is diffuse and biventricular.

The measurements of the nuclear and mitochondrial area confirm the nonspecific qualitative ultrastructural changes of hypertrophied myocardial cells as previously observed (14). The nuclear area and form factor results reflect a tendency of the nuclei to be enlarged and irregular in shape. Overall, the data on groups A and B are consistent with the figures of the first or adaptive stage of hypertrophy, as described by Ferrans (14) and the data of groups C and D are compatible with the figures of the second stage of hypertrophy (compensatory hyperfunction). None of the myocytes examined in this study showed the degenerative changes characteristic of the third stage of hypertrophy (exhaustion) as described by Ferrans.

Of what consequence might this hypertrophy be to the long-term survivors of cardiac transplantation? There is now increasing evidence (5-8) that the mere presence of myocardial hypertrophy may be dangerous. Left ventricular hypertrophy increases ectopic ventricular beats and predisposes to sudden death and acute myocardial infarction, independent of blood pressure and clinically obvious coronary artery atherosclerosis. This may be secondary to a lack of inadequate capillary proliferation in the hypertrophied heart with poor tissue oxygenation as a result (15). In cardiac transplant recipients who develop graft arteriosclerosis, (an incidence of almost 40% at 5 years) (2, 16), hypertrophy must be considered an ominous sign, for the combination of hypertrophy and coronary disease could severely jeopardize the myocardium. Furthermore, this is a challenging group of patients to follow clinically for evidence of coronary artery disease (3). The denervated heart has no afferent sensory fibers, and, therefore, graft arteriosclerosis appears as silent myocardial infarction, sudden death, or congestive heart failure rather than as angina pectoris. For this reason routine and frequent coronary arteriography is necessary in this group of patients.

Although some ischemic time is, at present, inevitable for all cardiac transplants, the results of this study and the known consequences of ventricular hypertrophy argue for the use of on-site donors whenever possible.

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GRAFT REJECTION IN RECIPIENTS OF T-CELL-DEPLETED HLA-NONIDENTICAL MARROW TRANSPLANTS FOR LEUKEMIA

IDENTIFICATION OF HOST-DERIVED ANTIDONOR ALLOCYTOTOXIC T LYMPHOCYTES[1]

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Clinical trials with bone marrow depleted of donor T lymphocytes indicate that both the incidence and severity of graft-versus-host disease (GVHD) in patients undergoing bone marrow transplantation (BMT) for treatment of leukemia are greatly reduced. However, there has been a concurrent increase in the incidence of graft rejection, particularly among recipients of HLA-nidentical marrow grafts. In order to investigate the nature of graft failure, peripheral blood mononuclear cells (PBMC) present at the time of graft failure have been characterized by phenotypic and functional analyses in 5 recipients of HLA-nonidentical marrow grafts. Rejection of HLA-nonidentical marrow grafts was associated with the emergence of host-derived T lymphocytes in all 5 patients. In 3 of these patients, the cells could be tested directly for cell-mediated cytotoxicity. Antidonor cytotoxicity was detected in each of these 3 patients. In one patient the target specificity of the cytotoxic lymphocytes was identified as the donor class I HLA antigen, HLA-B7. None of the patient PBMC mediated cytotoxicity against the natural killer cell target K562.

Graft versus host disease (GVHD)* is a major limitation to the success of bone marrow transplants (BMT) for the treatment of leukemia (1, 2). In several animal models, it has been demonstrated that the removal of T lymphocytes from donor bone marrow ameliorates or abrogates GVHD in the recipient animal (3–6). Preliminary clinical trials with bone marrow depleted of donor T lymphocytes indicate that both the incidence and severity of GVHD in engrafted patients undergoing BMT for treatment of leukemia are greatly reduced (7–12). Associated with these encouraging results, however, there has been a concurrent increase in the incidence of graft rejection, particularly among recipients of HLA-nonidentical marrow grafts (10). In a recent study of 42 patients with leukemia who

* Abbreviations used: ATG, antithymocyte globulin; BMT, bone marrow transplantation; CD, cluster differentiation; HLA, human leukocyte antigen; GVHD, graft-versus-host disease; PBMC, peripheral blood mononuclear cells.